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Temporal relationships between *Staphylococcus aureus* colonization, *filaggrin* expression, and pediatric atopic dermatitis

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Abstract

Background—Atopic dermatitis (AD) is characterized by *Staphylococcus aureus* (*S. aureus)* colonization. Longitudinal early life data delineating relationships of *S. aureus* colonization, barrier function, and AD outcomes are lacking. We define longitudinal *S. aureus* endotypes and AD pathogenesis in early life.

Methods—We defined longitudinal *S. aureus* skin colonization phenotypes across two annual visits (non-colonized: $V1^{-}V2^{-}$, early transient: $V1^{+}V2^{-}$, late onset: $V1^{-}V2^{+}$, persistent: $V1^{+}V2^{+}$) in the Mechanisms of Progression of Atopic Dermatitis to Asthma in Children cohort. We analyzed AD severity, sensitization, and skin barrier function across phenotypes, and performed mediation analyses between colonization and *FLG* expression.

Results—Persistent *S. aureus* colonization was associated with increased SCORAD at V1 (33.5 vs. 19.0, $p = 0.004$) and V2 (40.1 vs.16.9, $p < 0.001$), and lower non-lesional (NL) *FLG* at V2 (1.77 vs 4.09, $p = 0.029$) compared to the non-colonized phenotype, with early transient and late onset colonization as intermediate phenotypes. Children colonized at V2 demonstrated a decrease in NL-*FLG* expression from V1 to V2 compared to those non-colonized at V2 ($p=0.0012$), who maintained expression. This effect remained significant even after adjusting for V1 colonization and SCORAD ($p = 0.011$).

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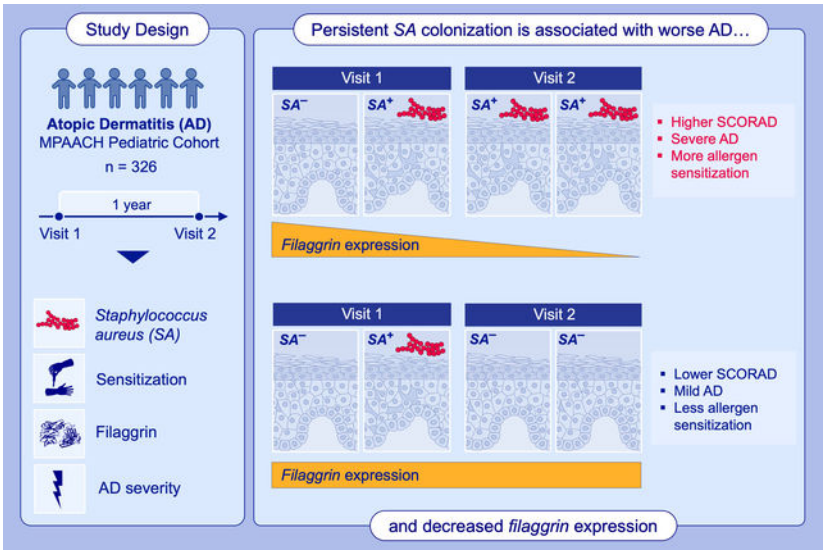
Conclusions—Our findings are the first to present longitudinal quantitative *FLG* expression and *S. aureus* skin colonization in early life and suggest that a decrease in NL-*FLG* drives later colonization. Hence, therapies to maintain NL-*FLG* expression may prevent *S. aureus* colonization. Further, a longitudinal AD endotype of persistent colonization is characterized by increased AD severity, sensitization, and decreasing NL-*FLG*.

Graphical Abstract



- We assessed *S. aureus* skin colonization and AD outcomes across two annual visits in the MPAACH cohort.
- Persistent *S. aureus* colonization is characterized by increased severity, allergic sensitization, and decreasing never-lesional *filaggrin* expression.
- Low never-lesional *filaggrin* expression positively mediates future *S.aureus* colonization. Maintaining *filaggrin* expression may be therapeutically important to halt colonization and worse AD outcomes.

Abbreviations: AD, atopic dermatitis; MPAACH, Mechanisms of Progression of Atopic Dermatitis to Asthma in Children; SA, *Staphylococcus aureus*; SCORAD, SCORing Atopic Dermatitis

Keywords

atopic dermatitis; filaggrin expression; longitudinal colonization; mediation analysis; *Staphylococcus aureus*

INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease that affects 15–30% of children and 2–10% of adults, and often precedes the development of allergic comorbidities.[1, 2] AD is characterized by a dysfunctional skin barrier, which may be

associated with deficiencies in the skin structural protein filaggrin (FLG), Th2 inflammation, skin microbial dysbiosis and exposure to exogenous irritants and/or allergens.[3]

Dysbiosis of the skin microflora with increased colonization by the gram-positive opportunistic bacterium *Staphylococcus aureus (S. aureus)* and the loss of commensal skin microbes is a hallmark of AD.[4] *S. aureus* exacerbates skin inflammation and dominates the microbiome during AD flares, thereby worsening AD severity.[5] Defects in the epidermal barrier worsen inflammation and further enhance entry and penetrance of *S. aureus* into the dermis.[6] Presence of FLG breakdown products, essential components of the natural moisturizing factor (NMF), has been shown to reduce *S. aureus* growth rate and cell density *in vitro.*[7] Further, *FLG* mutations are associated with reduced barrier integrity and increased *S. aureus* colonization on lesional skin *in vivo.*[6, 8] While this link between AD severity, FLG deficiency, and *S. aureus* colonization is increasingly recognized, studies to date have not examined *S. aureus* colonization and *FLG* expression over time in early life. [5, 9–11]
A recently convened workgroup of thought leaders in the field recognized that defining longitudinal phenotypic and endotypic subgroups of AD is a critical need.[12] Herein, we directly address this by leveraging the MPAACH (Mechanisms of Progression from AD to Asthma in Children) early-life cohort (n=326), which includes extensive longitudinal collection of clinical information and biospecimens[13]. We quantified skin *FLG* expression levels over time, defined longitudinal *S. aureus* skin colonization phenotypes and the impact on AD clinical outcomes, and then integrated these to explain the interplay between skin *FLG* and *S. aureus* colonization. The results from this study provide novel insights into key temporal relationships and drivers of *S. aureus* colonization in early life, a critical gap in current knowledge.

METHODS

Subjects

MPAACH is a prospective longitudinal early-life cohort of children with AD that has been previously described.[13,14] Eligible children were aged ≤3 years upon enrollment, had a gestation of ≥36 weeks, and either a diagnosis of AD (based on the Hanifin and Rajka Criteria for Atopic Dermatitis) or the parent(s)/legal authorized representative indicated a positive response to each of the 3 questions from the Children's Eczema Questionnaire (CEQ)[15]. Samples and specimens were collected at each annual visit. Children are instructed to stop using lotions, creams, and ointments on the skin the night prior to collection. This study was approved by the Institutional Review Board at CCHMC, and all subjects signed informed consent/parental permission prior to participation.

Keratinocyte Sampling and Identification of S. aureus

Lesional skin was defined by the Hanifin and Rajka diagnostic criteria for atopic dermatitis.[16] Non-lesional (NL) skin was defined as at least 10 cm from lesional (L) sites and had no history of lesions at that site according to parental report. SmartSolve skin tape strips measuring 1 inch x 1 inch were taken from L and NL-skin at each visit. Briefly, skin cells were sampled by placing the tape strip on the skin, gently massaging the tape for 15–20 seconds, removing the tape, and storing it in ice-cold in BL buffer supplemented with 2%

thio-glycerol (Promega, Madison, WI). This process was repeated to provide a total of 12 tape strips for each sampled skin site at each visit. RNA was extracted from tapes 8–9 using a two-stage process. The tapes were first extracted with a phenol-chloroform extraction and then the resulting RNA was purified using the ReliaPrep RNA cell mini prep kit following manufacturers guidelines (Promega). Complementary DNA was made with SuperScript IV VILO (ThermoFisher) and RT-qPCR reactions were carried out with the following Taqman gene expression assays: *18S* (Hs03003631_g1), *FLG* (Hs00856927_g1). *FLG* expression levels were normalized to *18S* levels. The maximum gene expression value from either tape strip 8 or 9 from each subject was used to denote *FLG* expression.

Sheep blood agar contact plates (Hardy Diagnostics) were used to collect host skin biome samples from L and NL-skin at each visit. Bacterial DNA was subjected to quantitative polymerase chain reaction (qPCR) targeting *femA-S. aureus* and *Universal 16S rRNA* to confirm *S. aureus* at either L or NL skin. Presence and absence of *femA* was determined based on the negative control at a Ct cutoff value of 30 on the ThermoFisher Cloud Connect online software. Phenotypes were determined by presence or absence of *S. aureus* by *femA* positive qPCR at either L or NL site at a visit. Detailed description of all methods and materials can be found in the Supporting Information.

RESULTS

S. aureus presence is associated with a more severe AD phenotype

There were 326 MPAACH subjects who had completed both V1 and V2 at the time of analysis. Bacterial isolates positive with the agglutination assay across V1 and V2 were further verified as *S. aureus* (*SA*) with *femA* qPCR to identify subjects with *S. aureus* presence or absence at each visit.

The subset of 326 subjects was initially categorized into two groups: non-colonized (SA-) and colonized (SA+) (Table 1). The SA+ group (19%) had *S. aureus* presence on L or NL skin at either V1 or V2 whereas the SA- group (81%) did not show *S. aureus* presence at V1 nor V2 on L or NL skin. At V1 and V2, the SA+ group had significantly higher SCORAD ($p = 0.008$, $p < 0.001$) and L-TEWL ($p < 0.001$, $p = 0.004$) and was more likely to have moderate/severe AD ($p = 0.007$, $p < 0.001$) and to be allergen-sensitized ($p = 0.035$, $p = 0.027$) compared to the SA- group. In addition, both L-*FLG* ($p = 0.022$) and NL-*FLG* ($p < 0.001$) expression at V2, but not V1 L or NL-*FLG* expression, were significantly lower in colonized subjects (Table 1). There were no baseline differences in age, sex, race, parental reported antibiotic usage in the past 12 months, steroid cream usage, early onset AD and environmental exposures between the two groups.

Persistent *S. aureus* colonization is associated with a more severe AD phenotype

Next, we further classified the colonization phenotypes to elucidate the temporal relationship between colonization and skin barrier function, sensitization, and clinical outcomes.
We defined four phenotypes, and their characteristics are summarized in Table 2: **non-colonized**: SA- at V1 and V2; **early transient**: SA+ at V1, but SA- at V2; **late onset**: SA- at V1, but SA+ at V2; **persistent**: SA+ at V1 and V2. There were no baseline differences in

demographics, parental reported antibiotic usage in the past 12 months, steroid cream usage, early onset AD or environmental exposures between the phenotypes.

There were significant differences in SCORAD across all phenotypes (V1: p = 0.012, V2: p < 0.001, Table 2). Children with persistent *S. aureus* colonization had a higher median SCORAD at V1 compared to non-colonized children (33.5 vs. 19.0, p = 0.004), and this difference was more pronounced at V2 (40.1 vs.16.9, p < 0.001). Children with early transient and late onset colonization had intermediate SCORAD values between non-colonized and persistent groups at both V1 and V2 (Table 2).

We next evaluated the proportion of subjects with mild vs moderate/severe AD between the *S. aureus* phenotypes at V1 and V2. Compared to the non-colonized phenotype, there was a higher proportion of children with moderate/severe AD at V2 in the early transient (53% vs. 24%, p < 0.001), late onset (53% vs. 24%, p = 0.028) and persistent (78% vs. 24%, p = 0.001) phenotypes (Fig 1A). Although there were differences in the proportion of subjects with mild vs moderate/severe AD among the *S. aureus* phenotypes at V1 (p = 0.045, Table 2), there were no significant differences across pairwise comparisons at V1 (Fig 1A).

We next evaluated allergen sensitization amongst the colonization phenotypes. At V1, there was a higher proportion of subjects who were allergen-sensitized in the persistent phenotype compared to the early transient phenotype (89% vs. 47%, p = 0.030) and non-colonized phenotype (88% vs. 42%, p = 0.012) (Fig 1B). This higher prevalence of sensitization continued at V2 in children with persistent *S. aureus* colonization compared to the non-colonized children (78% vs. 42.0%, p = 0.045). However, the relationships between sensitization patterns and *SA* phenotype may be dependent on AD severity as their association was not significant after controlling for AD severity.

Decreased non-lesional skin barrier integrity is associated with *S. aureus* colonization

We next evaluated *FLG* RNA expression and TEWL across the *S. aureus* phenotypes. At V2, NL-*FLG* expression was lower in the early transient (p = 0.017), late onset (p = 0.034) and persistent (p = 0.029) colonization phenotypes compared to the non-colonized group (Fig 1D). Further, the data showed a trend of decreasing NL-*FLG* expression from non-colonized to early transient, early transient to late onset, and late onset to persistent groups, suggesting a dose effect. At V1, the median NL-*FLG* expression was lower in the early transient compared to the late onset colonization phenotype (p = 0.012, Fig 1C), but no other differences were noted between the groups. At V1, L-*FLG* expression did not differ between the phenotypes whereas at V2, the early transient colonization group had higher L-*FLG* expression compared to the late onset colonization group (p = 0.013, Fig 1E–F). There was a higher L-TEWL at V1 in the early transient phenotype (p < 0.001, Fig S1A) and a higher NL-TEWL at V2 in the late onset phenotype (p = 0.042, Fig S1D) compared to the non-colonized phenotype. We next evaluated AD severity and skin barrier function at V1 and V2 within each phenotype (Table 3). The non-colonized group showed improvement in SCORAD (p<0.001) and NL-TEWL (p=0.013) over time. The V1 colonized only (early transient) showed no change in these parameters over time, but the V2 colonized only (late onset) showed a dramatic decrease in NL-*FLG* (p = 0.031), and the persistent colonized

group showed low barrier function and high SCORAD throughout both timepoints. These data validated our key findings across all groups.

V2 *S. aureus* colonization is associated with a decrease in non-lesional *filaggrin* expression over time

To further study temporal associations between *S. aureus* colonization and *FLG* expression, subjects were dichotomized into V2 SA+ (orange line) and V2 SA- (blue line) groups. The L-*FLG* and NL-*FLG* expression values at each visit were plotted, and linear mixed-effect model equations were calculated for each group (Fig 2). There was a significant difference in the change or delta in NL-*FLG* expression level from V1 to V2 based on V2 *S. aureus* colonization ($p = 0.0012$, Fig 2A). Specifically, children who were colonized with *S. aureus* at V2 (V2 SA+) demonstrated a 0.98 unit decrease in NL-*FLG* expression over time on average, while those non-colonized at V2 (V2 SA-) had a 0.03 unit increase in their NL-*FLG* over time. To determine the impact of colonization status at V1 on these observations, we further stratified by V1 SA status (V1 SA+ and V1 SA-) (Fig 2B–C). The decrease in NL-*FLG* expression from V1 to V2 in those colonized at V2 was observed in both V1 SA+ and SA- groups, although the V1 SA+ group reached only nominal significance. Similarly, the increase in NL-*FLG* expression from V1 to V2 in those non-colonized at V2 was observed in both V1 SA+ and SA- groups. These results suggested that the NL-*FLG* expression over time determines colonization at V2 and that this is independent of colonization at V1. To determine whether this was the case, we directly examined whether the change in NL-*FLG* expression (V2 – V1 NL-*FLG*, denoted as delta NL-*FLG*) was associated with *S. aureus* colonization status. V2, but not V1 colonization, was significantly associated with decreased delta NL-*FLG* (V1 colonization: $p = 0.56$; V2 colonization: $p = 0.010$). This relationship between V2 colonization and delta NL-*FLG* remained significant after controlling for V1 *S. aureus* colonization status and V1 SCORAD ($p = 0.011$). There were no significant differences in L-*FLG* expression over time irrespective of colonization status (Fig 2D–F).

Non-lesional *filaggrin* expression mediates *S. aureus* colonization

We developed a mediation model to examine the causal relationship between *S. aureus* status and *FLG* expression. In the model, V1 *S. aureus* status was the independent variable, V2 *S. aureus* status was the dependent variable, and V2 NL-*FLG* expression was the mediator. Our sample size was too small to evaluate delta NL-*FLG* as the mediator. The direct effect of V1 *S. aureus* on V2 *S. aureus* status was significant (ADE average = 0.144, $p = 0.027$). V1 *S. aureus* status was also marginally associated with lower V2 NL-*FLG* ($\beta = -0.30$, $p = 0.09$). We observed a mediating effect of V2 NL-*FLG* expression levels on V2 *S. aureus* colonization (ACME average = 0.046, $p = 0.046$).

This mediation model focused on a unit-change in the expression levels. As differences in *FLG* expression among children often did not exceed 1 unit, interpretation of the effect sizes can be challenging. Thus, we compared the higher and lower *FLG* expression tertiles ($n = 162$). The mediation effect of V2 NL-*FLG* expression on V2 *S. aureus* status was significant (ACME average = 0.05, $p = 0.018$) but the direct effect was not (ADE average = 0.073, $p = 0.28$). When evaluating the components of the mediation, we found that lower

V2 NL-*FLG* is associated with 11.04 times the odds of being colonized by *S. aureus* at V2 (p = 0.024) compared to higher *FLG* expression. We also found that V1 SA+ children had 3.32 times the odds of having lower V2 NL-*FLG* (p = 0.017) compared to V1 SA- children, which is consistent with the patterns of NL-*FLG* seen in Figure 1. However, the presence of a significant mediation term supports that V2 NL-*FLG* expression contributes to V2 colonization risk above the direct risk of V1 colonization status. These data show that while NL-*FLG* expression mediates *S. aureus* colonization at V2 independent of V1 colonization, this effect is likely modified by V1 colonization status. Indeed, when we evaluated the effect size of the ACME model conditional on V1 *S. aureus* status, we observed that *S. aureus* positive group had approximately twice the effect size for our mediation model. Formal interaction between V1 *S. aureus* colonization status and V2 NL-*FLG* expression was not significant (p = 0.26). The main effect of V2 NL-*FLG* on V2 *S. aureus* colonization remained significant even after adjustment for V1 and V2 SCORAD, race, and age, supporting the robustness of our findings (data not shown).

To evaluate the specificity of the mediation effect, we evaluated additional mediation models. We considered V1 NL-*FLG* and V1/V2 L-*FLG* expression as mediators, but these were not significant (data not shown). Lastly, to evaluate whether changes in *FLG* expression were mediated by *S. aureus* colonization, we developed another mediation model to study the relationship between V1 NL-*FLG* expression as the independent variable, V2 NL-*FLG* expression as the dependent variable, and V2 *S. aureus* status as the mediator. The mediation effect was not significant (Table S1).

DISCUSSION

This is the first study to report longitudinal quantitative skin *FLG* expression levels and *S. aureus* skin colonization data in children with AD over time in early life. By leveraging the MPAACH prospective early-life cohort of children with AD, we defined longitudinal *S. aureus* skin colonization phenotypes, determined skin L- and NL-*FLG* expression trajectories over time, and then integrated these to understand the interplay of skin *FLG* levels and *S. aureus* colonization and their impact on sensitization patterns and AD clinical outcomes. Our data revealed that V2 NL-*FLG*, but not L- *FLG,* expression levels mediated V2 *S. aureus* colonization independent of V1 and V2 AD severity, underscoring the critical role that the NL skin plays in AD pathogenesis. The effect was modified by V1 *S. aureus* colonization status such that children colonized at V1 had increased odds of having lower V2 NL-*FLG* compared to children without *S. aureus* colonization at V1. Children colonized with *S. aureus* at V2 showed a decrease in NL-*FLG* expression over time while those who were V2 SA- maintained or slightly increased NL-*FLG* expression. Persistent colonization was associated with increased sensitization and worsened AD severity as assessed by SCORAD. Our findings reveal a longitudinal AD endotype of persistent *S. aureus* colonization characterized by increased AD severity, allergic sensitization, and decrease in NL-*FLG* expression. Collectively, these data strongly suggest that intervention designed to interrupt and change the decrease in NL-*FLG* expression early in life may be critical in blocking later *S. aureus* colonization and the clinical sequalae.

V2 *S. aureus* colonization was directly mediated by V2 NL-*FLG* expression demonstrating the contribution of NL-skin barrier disruption to late onset and persistent colonization. FLG breakdown products have been reported to lead to increased acidification of media, decreased density of *S. aureus* and decreased production of bacterial colonization and immune evasion proteins in an *in vitro* model[7], so a decrease in *FLG* expression could impact future colonization. Another study showed increased *S. aureus* adherence with either *FLG*-knock down or supplementation of IL-31, a cytokine which has been shown to downregulate *FLG* expression in epidermal skin models.[17,18] An additive effect was observed with both *FLG*-knock down and IL-31 supplementation.[18] Therefore, lower FLG may create a permissive environment in the context of attenuated immunity for further colonization. In a recent pediatric study, skin *S. aureus* load in children with difficult-to-treat AD was not associated with the *FLG* mutation status,[19] but they did not examine the expression level of *FLG*, which is affected by more than genetics. This underscores a major strength of our study, where we quantified *FLG* RNA expression levels in NL and L skin over time. We did not observe significance when using V1 or V2 L-*FLG* expression as mediators in our analyses, indicating that the link between *FLG* expression and colonization may be restricted to NL-skin. This reinforces previous findings from our group and others that NL-skin is driving the AD and sensitization phenotype.[13, 20] This mediation, however, does not prove causality and does not rule out the contribution of other potential factors such as medication and inflammation in *S. aureus* colonization. While we didn't see a mediating effect by *S. aureus* colonization on NL-*FLG* expression, it remains possible and even likely that *S. aureus* colonization also acts upstream leading to further decreased *FLG*, setting up a detrimental feed forward loop. For example, *S. aureus* superantigen B (SEB) has been shown to increase IL-31 expression and suppress *FLG* expression.[21] Medications could also impact our findings. While we did not observe differences in the use of parental reported use of antibiotics in the past 12 months and steroid cream usage at baseline between the colonization phenotypes, consistent use of medications including antibiotics and biologics may affect colonization rates and therefore, *FLG* expression.

We observed that V2 SA+ children had a decrease in NL-*FLG* expression from V1 to V2 compared to those who were V2 SA-, who maintained similar *FLG* levels over time. This decreae in NL-*FLG* expression was not conditional on V1 colonization status but likely driven by the V1 SA- group, as the magnitude of the decrease is more pronounced in this group. Our linear regression models reinforced that changes in NL-*FLG* are associated with V2, but not V1, *S. aureus* colonization, suggesting that the change is important for determining future colonization for the late onset and persistent phenotypes in our sample. A previous study demonstrated sustained change in *FLG* expression within weeks in response to treatment[22]. Maintenance of NL-*FLG* expression levels in V2 SA- children may be due to numerous factors including better compliance with skin care regimens for eczema treatment which may lead to improved barrier function and/or decreased inflammation. Th2 inflammation has been shown to lead to reduced skin *FLG* expression.[23] As such, a decrease in local inflammation would be predicted to increase *FLG* expression and overall skin barrier health.[24] In contrast, persistent inflammation and/or poor skin care may lead to sustained decreased *FLG* expression over time.

Persistent colonization over time was associated with a higher SCORAD and increased AD severity and proportion of children with sensitization. Studies have shown that *S. aureus* is correlated with increased expression of proinflammatory cytokines including TSLP, IL-8, IL-1β from keratinocytes, mast cell degranulation and increased Th2-mediated inflammation.[25] As such, differences in an individual's ability to clear *S. aureus* colonization likely also plays a role in AD outcomes.

To our knowledge, this study is the first to examine the relationship between longitudinal *FLG* expression and *S. aureus* colonization. Two related studies from 2012 and 2017 performed 16S rRNA sequencing and shotgun sequencing on skin tapes on 422 pediatric samples across baseline, flare and post-flare periods but neither investigated skin barrier function.[5, 11] Other studies have examined strain variation over time and association with AD severity.[9, 26, 27] Another recent study of 49 AD and control adults identified distinct microbial configurations ("dermotypes") identified by shotgun metagenomics across two visits separated by 4 weeks. Although samples were collected to examine components of the NMF over time, their analysis was restricted to visit 1, where they found no difference between the NMF protein quantities between the dermotypes.[28] While we also showed an association between colonization and AD severity, the mediating effect of NL-*FLG* on colonization remained significant independent of V1 and V2 SCORAD.

The strengths of this study lie in the cohort size and extensive biospecimen and data collection capabilities of MPAACH, but it is not without limitations. By design, all participants in this MPAACH cohort have atopic dermatitis, so we cannot make conclusions regarding AD development. However, we did not observe any differences when examining early onset AD (AD appearance at or before 3 months of age) vs late onset (AD appearance after 3 months of age) between the non-colonized vs colonized groups and between the phenotypes. The study may also be limited by inherent variability in direct sampling of skin via agar plates versus other sampling methodologies. Approximately 20% of the subjects in this study were colonized at either visit. This is in line with a previous study utilizing skin swabs which identified *S. aureus* positive axillar swabs in approximately 15% of infants at the time of AD onset.[29] Importantly, variability in sampling methodologies, locations, downstream analysis of biospecimen, *S. aureus* biofilm production, and ages of participants yield varying results on colonization rates.[30] Sequencing may overrepresent microbes that are present in very low quantities whereas contact plates methodology utilized herein may be useful in directly sampling *S. aureus* isolates that are most relevant to disease progression. We also utilized a multi-layered approach to ensure that our results are specific to *S. aureus,* which could lead to lower colonization rates compared to other studies. Skin barrier function may also be determined by lipids and other structural proteins such as loricrin and claudin, which we intend to investigate in future analyses. Lastly, the prevalence of commensal microbes that may affect *S. aureus* colonization was not assessed. However, metagenomic studies on skin tape collected from L- and NL-skin at each MPAACH visit will allow us to determine the taxonomic, strain and functional profiles of the microbiome over time in the cohort.[31]

Our findings regarding the temporal relationship between *FLG* expression and *S. aureus* colonization in early life have significant clinical implications. Given the relationship

between persistent colonization and worse clinical outcomes, preventing or disrupting colonization is an objective of therapies. Broad-range antimicrobial therapies exhibit minimal benefits and unintended side effects, highlighting the need for other personalized therapeutic strategies.[32] Current AD therapies mostly target L-skin and are aimed at decreasing inflammation or increasing skin moisture, but there are no strategies that specifically target *FLG* expression in early life in NL-skin. Our findings suggest that it may be clinically useful to monitor skin *FLG* levels over time and optimize therapies aimed at inhibiting future and persistent *S. aureus* colonization in early life to prevent worsening AD and progression to other comorbidities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ACME	average causal mediation effect
AD	atopic dermatitis
ADE	average direct effect
CCHMC	Cincinnati Children's Hospital Medical Center
FLG	filaggrin
L/NL	lesional/non-lesional
MPAACH	Mechanisms of Progression of Atopic Dermatitis (AD) to Asthma in Children
MSA	Mannitol Salt Agar
OR	odds ratio
PCR	polymerase chain reaction
SA	*Staphylococcus aureus*
SCORAD	SCORing Atopic Dermatitis index
SPT	skin prick test
TEWL	Trans-epidermal water loss

V1	Visit 1
V2	Visit 2

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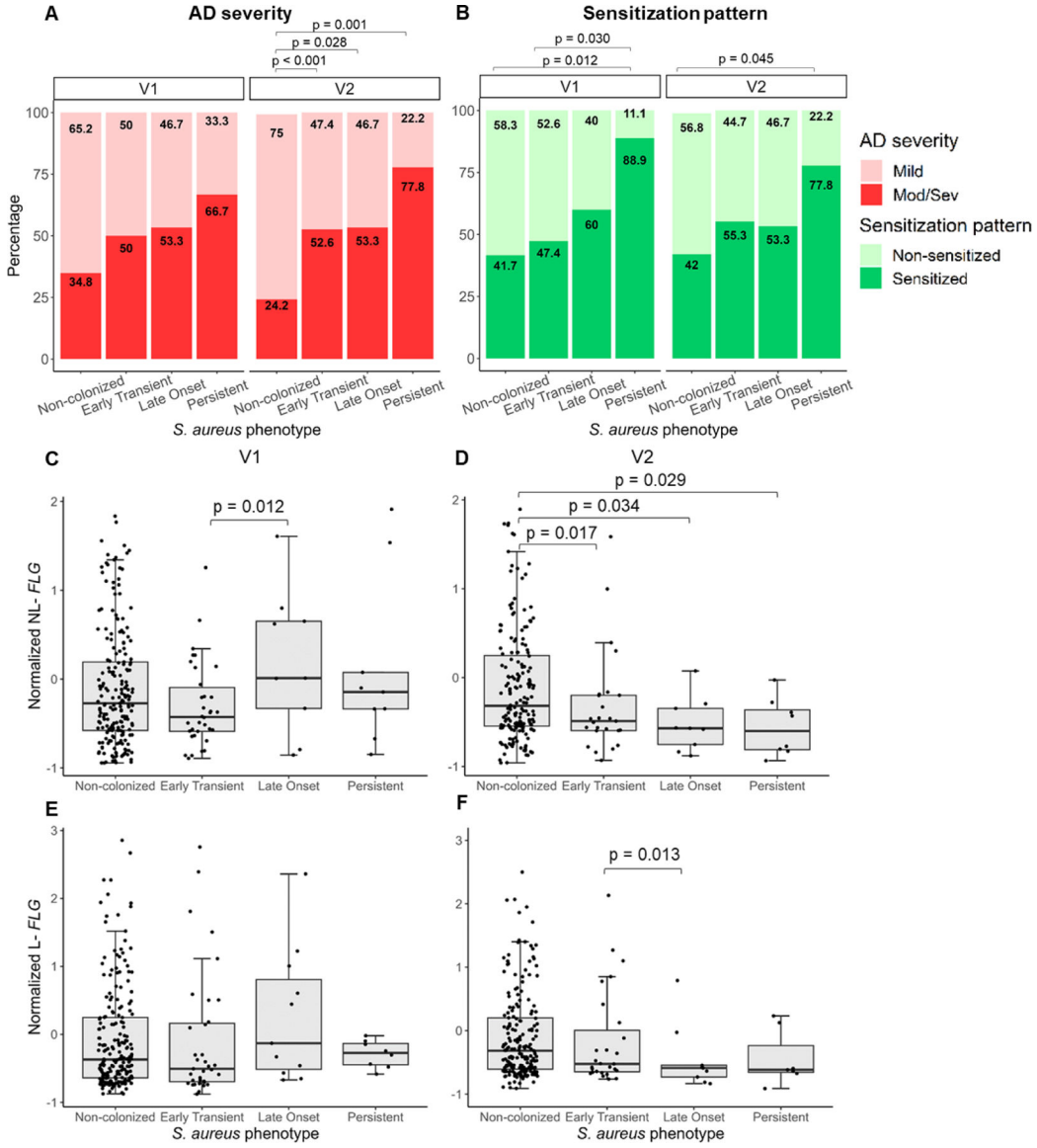

Fig 1. AD severity, sensitization status and *FLG* expression across *S. aureus* phenotypes in MPAACH subset.

(A) AD severity **(B)** sensitization pattern **(C,D)** non-lesional (NL) and **(E,F)** lesional (L) *FLG* at V1 and V2 across four *S. aureus* phenotypes. Differences across phenotypes were evaluated using Kruskal-Wallis test, followed by Dunn's test where significance was observed. Comparisons of mild vs. moderate-to-severe (Mod/Sev) AD and non-sensitized vs. sensitized groups between phenotypes were done using Chi-square test. Outliers were removed in the figures for better visualization.

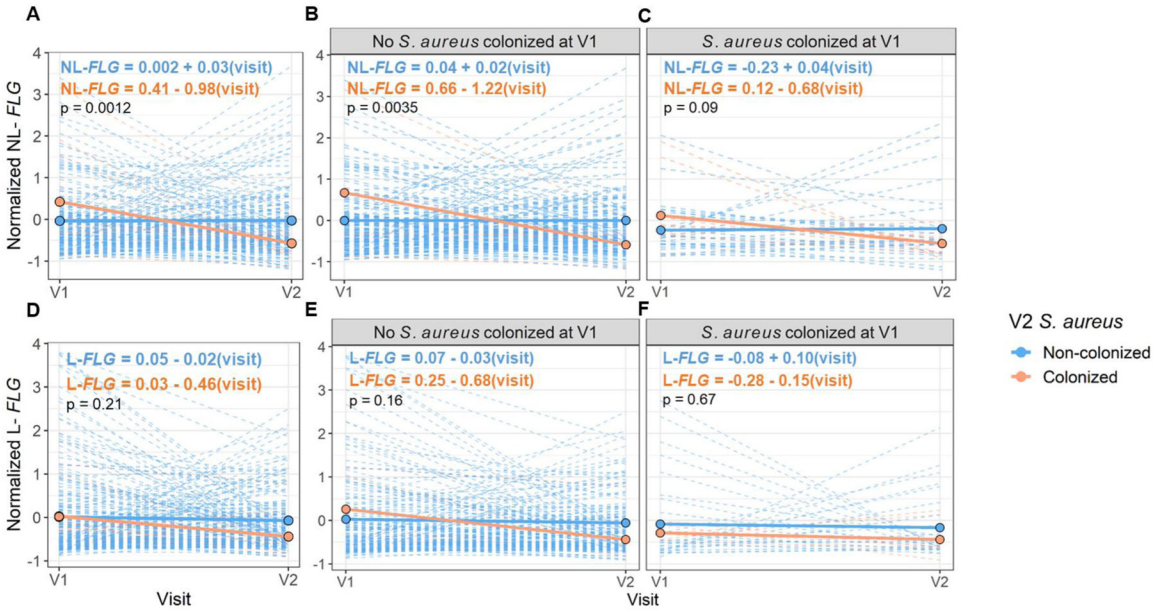

Fig 2. *FLG* expression level and *S. aureus* colonization across V1 and V2.

(A) Decreasing trend of NL-*FLG* expression in participants with V2 *S. aureus* colonization, **(B,C)** stratified by V1 *S. aureus* colonization. **(D)** L-*FLG* expression from V1 to V2 in participants with V2 *S. aureus* colonization, **(E,F)** stratified by V1 *S. aureus* colonization. Model equations were constructed using linear mixed-effect model to account for the random subject effect, fixed effects of V2 *S. aureus* colonization and time (i.e., visit), and an interaction term between colonization and time. P-values represent the significance of interaction terms. Season-adjusted *FLG* expression values are presented. Dashed lines represent data on the individual scale; solid lines represent the mean for each group.

Table 1.

Characteristics of MPAACH cohort by *S. aureus* colonization[†]

***S. aureus* colonization**	**Sample size for each variable**	**Non-colonized n = 264 (81.0)**	**Colonized n = 62 (19.0)**	***p*-value[‡]**
Demographic				
Age (y)	326	2.1 (1.5–2.4)	1.9 (1.5–2.4)	0.42
Male sex	326	137 (52)	35 (56)	0.52
Black race	326	180 (68)	35 (56)	0.08
Early onset AD (<3 mos of age)	326	111 (42)	31 (50)	0.25
AD Severity at Visit 1				
SCORAD (score)	326	19.0 (13.0–29.1)	27.9 (12.7–41.5)	**0.008**
Severity group	326			**0.007**
Mild (SCORAD < 25)		172 (65)	29 (47)	
Moderate/Severe (SCORAD ≥ 25)		92 (35)	33 (53)	
AD Severity at Visit 2				
SCORAD (score)	324	16.9 (9.6–24.7)	26.2 (11.2–42.9)	< **0.001**
Severity group	324			< **0.001**
Mild (SCORAD < 25)		198 (75)	27 (43)	
Moderate/Severe (SCORAD ≥ 25)		64 (24)	35 (57)	
Skin barrier function at Visit 1				
Lesional skin				
Keratinocyte *FLG* expression[§]	267	2.36 (0.68–6.05)	1.95 (0.54–5.14)	0.56
TEWL ($g/m^2/h$)	324	11.5 (8.4–18.3)	16.7 (11.3–28.9)	< **0.001**
Non-lesional skin				
Keratinocyte *FLG* expression[§]	268	3.97 (1.21–8.83)	3.31 (1.72–7.79)	0.76
TEWL ($g/m^2/h$)	323	9.4 (7.1–13.2)	9.6 (8.0–12.9)	0.32
Skin barrier function at Visit 2				
Lesional skin				
Keratinocyte *FLG* expression[§]	241	2.89 (1.12–8.37)	1.13 (0.30–4.45)	**0.022**
TEWL ($g/m^2/h$)	314	11.7 (8.2–16.2)	17.9 (8.8–24.2)	**0.004**
Non-lesional skin				
Keratinocyte *FLG* expression[§]	242	4.09 (1.48–9.00)	1.65 (0.48–4.48)	< **0.001**
TEWL ($g/m^2/h$)	312	9.2 (7.1–11.5)	9.7 (7.6–13.6)	0.17
Sensitization at Visit 1				
Sensitization group	326			**0.035**
Nonsensitized		154 (58)	27 (43)	
Sensitized		110 (42)	35 (57)	
Total serum IgE	236	32 (10–93)	46 (13–228)	0.13
Sensitization at Visit 2				
Sensitization group	323			**0.027**
Nonsensitized		150 (57)	26 (42)	

***S. aureus* colonization**	**Sample size for each variable**	**Non-colonized n = 264 (81.0)**	**Colonized n = 62 (19.0)**	***p*-value[‡]**
Sensitized		111 (42)	36 (58)	
Total serum IgE	204	70 (18–142)	118 (30–408)	0.053

[†]Data are presented in median (IQR) or *n* (%).

[‡]Comparison between *S. aureus* colonization groups were done using Wilcoxon rank-sum test for continuous variables, Chi-square tests for categorical variables.

[§]Season-adjusted *FLG* expression values were used in the comparison.

Table 2.

Characteristics of MPAACH cohort by *S. aureus* phenotypes[†]

***S. aureus* phenotype**	**Sample size for each variable**	**Non-colonized n = 264 (81.0)**	**Early Transient n = 38 (11.6)**	**Late Onset n = 15 (4.6)**	**Persistent n= 9 (2.8)**	***p*-value[‡]**
Demographic						
Age (y)	326	2.1 (1.5–2.4)	1.9 (1.5–2.4)	1.8 (1.4–2.4)	2.1 (1.9–2.4)	0.65
Male sex	326	137 (52)	21 (55)	10 (67)	4 (44)	0.66
Black race	326	180 (68)	22 (58)	8 (53)	5 (56)	0.36
Early onset AD (<3 mos of age)	326	111 (42)	16 (42)	10 (67)	5 (56)	0.26
AD Severity at Visit 1						
SCORAD (score)	326	19.0 (13.0–29.1)	25.3 (12.5–42.9)	27.3 (11.5–37.4)	33.5 (22.8–44.4)	**0.012**
Severity group	326					**0.045**
Mild (SCORAD < 25)		172 (65)	19 (50)	7 (47)	3 (33)	
Moderate/Severe (SCORAD ≥ 25)		92 (35)	19 (50)	8 (53)	6 (67)	
AD Severity at Visit 2						
SCORAD (score)	324	16.9 (9.6–24.7)	26.0 (10.2–36.6)	25.1 (12.0–37.7)	40.1 (28.0–68.8)	**<0.001**
Severity group	324					**<0.001**
Mild (SCORAD < 25)		198 (75)	18 (47)	7 (47)	2 (22)	
Moderate/Severe (SCORAD ≥ 25)		64 (24)	20 (53)	8 (53)	7 (78)	
Skin barrier function at Visit 1						
Lesional skin						
Keratinocyte *FLG* expression[§]	267	2.36 (0.68–6.05)	1.23 (0.22–5.10)	3.50 (1.29–8.28)	2.26 (1.87–3.18)	0.35
TEWL (g/m^2/h)	324	11.5 (8.4–18.3)	19.6 (11.3–32.1)	14.7 (12.5–19.5)	15.3 (11.4–19.7)	**0.002**
Non-lesional skin						
Keratinocyte *FLG* expression[§]	268	3.97 (1.21–8.83)	2.27 (1.22–6.52)	11.49 (4.36–15.09)	4.06 (2.28–9.35)	0.10
TEWL (g/m^2/h)	323	9.4 (7.1–13.2)	9.4 (8.0–12.4)	9.6 (7.7–13.7)	11.4 (9.2–14.5)	0.62
Skin barrier function at Visit 2						
Lesional skin						
Keratinocyte *FLG* expression[§]	241	2.89 (1.12–8.37)	2.09 (0.68–4.68)	0.81 (0.20–2.89)	0.26 (0.15–2.64)	0.06
TEWL (g/m^2/h)	314	11.7 (8.2–16.2)	16 (8.5–22.1)	18.3 (11.7–24.2)	20.5 (8.7–45.1)	**0.022**
Non-lesional skin						

***S. aureus* phenotype**	**Sample size for each variable**	**Non-colonized n = 264 (81.0)**	**Early Transient n = 38 (11.6)**	**Late Onset n = 15 (4.6)**	**Persistent n= 9 (2.8)**	***p*-value[‡]**
Keratinocyte *FLG* expression[§]	242	4.09 (1.48–9.00)	1.65 (0.47–4.60)	2.13 (0.49–3.61)	1.77 (0.46–2.79)	**0.004**
TEWL (g/m^2/h)	312	9.2 (7.1–11.5)	9.7 (7.6–13.4)	10.2 (7.8–13.1)	9.6 (7.7–14.2)	0.59

[†]Data are presented in median (IQR) or *n* (%).

[‡]Comparison across *S. aureus* phenotypes are done using Kruskal-Wallis test for continuous variables, Chi-square tests for categorical variables. P-values of variables with small sample size (i.e. cell size of 0) are not reported.

[§]Season-adjusted *FLG* expression values were used in the comparison.

Table 3.

Comparison of V1 and V2 SCORAD, sensitization and skin barrier function in different *S. aureus* colonization/phenotype group[†]

***S. aureus* colonization/phenotype**	**Non-colonized (n = 264)**			**Early Transient (n = 38)**		
	V1	**V2**	***p*‡**	**V1**	**V2**	***p*‡**
AD severity						
SCORAD (score)	19.0 (13.0–29.1)	16.9 (9.6–24.7)	< **0.001**	25.3 (12.5–42.9)	26.0 (10.2–36.6)	0.12
Moderate/Severe AD (SCORAD ≥ 25)	92 (35%)	64 (24%)	**0.002**	19 (50%)	20 (53%)	0.99
Sensitization						
Sensitized	110 (42%)	111 (42%)	0.99	18 (47%)	21 (55%)	0.45
Total serum IgE	32 (10–93)	70 (18–142)	< **0.001**	44 (11–195)	62 (14–282)	0.20
Skin barrier - Lesional skin						
Keratinocyte *FLG* expression[§]	2.36 (0.69–6.05)	2.89 (1.12–8.37)	0.33	1.23 (0.22–5.10)	2.09 (0.68–4.68)	0.92
TEWL (g/m^2/h)	11.5 (8.4–18.3)	11.7 (8.2–16.2)	0.25	19.6 (11.3–32.1)	16.0 (8.5–22.1)	0.15
Skin barrier - Non-lesional skin						
Keratinocyte *FLG* expression[§]	3.97 (1.21–8.83)	4.09 (1.48–9.00)	0.67	2.27 (1.22–6.52)	1.65 (0.47–4.60)	0.58
TEWL (g/m^2/h)	9.4 (7.1–13.2)	9.2 (7.1–11.5)	**0.013**	9.4 (8.0–12.4)	9.7 (7.6–13.4)	0.78
***S. aureus* colonization/phenotype**	**Late Onset (n = 15)**			**Persistent (n = 9)**		
	V1	**V2**	***p*‡**	**V1**	**V2**	***p*‡**
AD severity						
SCORAD (score)	27.3 (11.5–37.4)	25.1 (12.0–37.7)	0.60	33.5 (22.8–44.4)	40.1 (28.0–68.8)	0.42
Moderate/Severe AD (SCORAD ≥ 25)	8 (53%)	8 (53%)	0.99	6 (67%)	7 (78%)	0.99
Sensitization						
Sensitized	9 (60%)	8 (53%)	0.99	8 (89%)	7 (78%)	0.99
Total serum IgE	64 (14–170)	144 (72–430)	0.56	176 (14–250)	93 (55–335)	0.50
Skin barrier - Lesional skin						
Keratinocyte *FLG* expression[§]	3.50 (1.29–8.28)	0.81 (0.20–2.89)	0.31	2.26 (1.87–3.18)	0.26 (0.15–2.64)	0.69
TEWL (g/m^2/h)	14.7 (12.5–19.5)	18.3 (11.7–24.2)	0.79	15.3 (11.4–19.7)	20.5 (8.7–45.1)	0.36
Skin barrier - Non-lesional skin						
Keratinocyte *FLG* expression[§]	11.49 (4.36–15.09)	2.13 (0.49–3.61)	**0.031**	4.06 (2.29–9.36)	1.77 (0.46–2.79)	0.15

***S. aureus* colonization/phenotype**	**Non-colonized (n = 264)**			**Early Transient (n = 38)**		
	V1	**V2**	***p*** ‡	**V1**	**V2**	***p*** ‡
TEWL ($g/m^2/h$)	9.6 (7.7–13.7)	10.2 (7.8–13.1)	0.79	11.4 (9.2–14.5)	9.6 (7.7–14.2)	0.65

†Data are presented in median (IQR) or *n* (%).

‡Comparison between V1 and V2 within each population were done using Wilcoxon signed rank test for continuous variables, McNemar's tests for categorical variables.

§Season-adjusted *FLG* expressions were used in the comparison.